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Evaluation of bioflocculants produced by some bacteria and their use to remove artificial turbidity from water

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Abstract

Bioflocculants offer a number of advantages over chemical flocculants for wastewater treatment. They are more environmentally friendly, safer, and often low cost-effective. The flocculating activity of bioflocculants to remove turbidity at a dose 4 ml were 45% ,27.19% ,41.19% and 44.28 % by bacterial species *Escherichia coli, Arcobacter cloacae, Aeromonas simiae, Exiguobactirum profundum* respectively while, at a dose 8 ml were 49.65%, 31% ,64 47% and 63.71. The flocculating activity of bioflocculants at (100/40) speed to remove turbidity by bacteria *Escherichia coli, Arcobacter cloacae, Aeromonas simiae* and *Exiguobactirum profundum* were 47.94% ,37.1%, 66.63% and 62.89% respectively while at (200/40) speed was 48%, 35.5%, 62.89% and 65.12%. The flocculating activity at a mixing time (4 min) to remove turbidity was 51.19%, 32.7%, 70.28% and 67.80% while, at 6 min was 41.79%, 34.98%, 71.67% and 70.0%. The result exhibited two bacteria *Aeromonas simiae and Exiguobactirum profundum* have the best efficiency in removing turbidity when tested on of synthetic turbid water in the laboratory. There for the bioflocculants have potential activity for industrial wastewater treatment.

Keywords: Bioflocculants, kaolin, flocculating activity, removing turbidity
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Introduction

Water is important resource for life however, water quality is constantly deteriorating due to increased contamination from industrial activities and urbanization **(**Gayathiri *et al*., 2022**).** Water pollution affects more than 1.2 billion people globally, leading to poor economic growth and food insecurity **(**Ma *et al*., 2008). Many industries, including pulp and paper and aquaculture industries, release high turbid effluent without proper treatment (Bonisławska *et al*., 2019; Grenda *et al*., 2020). Flocculants are mainly used in wastewater treatment because of their availability at low cost and their high flocculating abilities (Okaiyeto *et al*., 2016).

Flocculants can be divided into natural (chitosan, bioflocculant), inorganic ($Al_2(SO_4)_3$ and polyaluminum chloride) and organic synthetic (polyacrylic acid, polyacrylamide derivatives) flocculants. Many of organic and inorganic flocculants are used widely because low cost and high activity. However, the monomers and derivatives of both flocculants cause serious diseases, like neurological, cancer and Alzheimer's disease (Kurniawan *et al*. ,2020). Coagulation-flocculation is a chemical and physical process that is widely used to separate solids from wastewater. It is

one of the most common wastewater treatment methods (Hassimi *et al*., 2020; Maruyama *et al*.,2022). The principle of coagulation and flocculation is to increase the settling velocity of the pollutants, which naturally can take hours and even years for colloidal particles (Sun *et al*., 2021). Bioflocculants are substances produced by bacteria and fungi as they grow, using microbial technology, fermentation, and extraction. Composition of bioflocculants could be polysaccharides, proteins, nucleic acids, cellulose, sugar, or poly amino acids (Ugbenyen, *et al*., 2015; Kurniawan *et al*., 2022).

Several bioflocculant-producing bacterial species have been reported for their significant flocculating activities (>70–90%), yield, and various additional functions in wastewater treatment (Ben Rebah *et al*., 2018; Bisht *et al*., 2019). A lot of studies reported the production of bioflocculants compounds from various bacteria isolated from different sources, these species including *Citrobacter youngae* GTC 01314 from laboratory culture (Hatta *et al*., 2021), *Bacillus agaradhaerens* C9 was isolated from lake area (Liu *et al*., 2019), *Streptomyces platensis* was isolated from dam area (Agunbiade *et al*., 2018), *Bacillus pumilus* JX860616 was isolated from coastal area (Ngema *et al*., 2020) and *Pseudomonas aeruginosa* strain IASST201 was isolated from activated sludge (Pathak *et al*., 2017). The aim of the current study is to evaluate the performance of the bioflocculants produced by four bacterial isolates in removing turbidity of synthetic turbid water was tested at different concentrations, as well as choosing different speeds and times using the Jar test device.

Materials and methods

Isolation and identification of bacteria

The bacteria that are utilized in current study were isolated in previous study (AL khafaji *et al.,* 2023), isolates (A1, A3 and A5) form the Hamden wastewater treatment plant and isolates (D) from contaminated soil by oil in Rumelia area north of Basrah city. The bacterial isolates were characterized by adopting an analysis of 16S rDNA gene. The PrestoTM Mini g DNA bacteria kit from the Gene aid company was used to isolate the bacterial DNA. The polymerase chain reaction was used to amplify the 16S rDNA gene using primers 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5- GGTTACCTTGTTACGACTT-3). The Polymerase chain reaction (PCR) program for amplifying the target 16S rDNA gene was an initial denaturation of 96°C for 3 min, 27 cycles including 96 °C for 30s, annealing 56 ºC for 25 s and elongation temperature at 72 °C for 15 s and final elongation at 72 °C for 10 min (Miyoshi *et al*., 2005). The purification and sequencing of PCR products were performed by Macrogen company (South Korea). The proofreading of the obtained 16S rDNA gene sequences was conducted by utilizing chromas, the sequences were compared with NCBI nucleotide sequences using BLAST tools to determine the sequence similarity.

Preparation kaolin clay solution

The flocculation experiments to test bacteria as bioflocculant producer were done with synthetic turbid water which prepared by mixing kaolin powder with water. 500 NTU concentration of synthetic turbid water was prepared by mixing kaolin1.5g with distilled water 3L in beaker and stirred for 30 min to be homogeneous to simulate an initial turbidity concentration of 500 ± 50 NTU (Ahmad *et al*., 2022).

Application of bioflocculant-producing bacteria in kaolin clay Suspension

After the identification, the selected four bacterial species were used to evaluate the performance of the produced bioflocculants for removing turbidity, the supernatant of the selected bacterial species was tested at different concentrations, as well as choosing

of different speeds and times using the jar test device. An artificial turbidity was prepared in the laboratory by mixing 1.5 Kaolin clay in 3.5 L of distilled water to reach a turbidity of 500 (NTU). The operational conditions of 500 mL total volume in 500 mL beaker glass rapid mixing speed of 100 rpm for 4 min and 6 min, and rapid mixing speed of 200 rpm for 4 and 6 min and slow time with 40 rpm, 15 mL of 1% $CaCl_2$ with variation concentration of bioflocculants (4 mL, 8 mL) were applied during this stage (Adnan *et al.,* 2017). After the two options of rapid and slow mixing of flocculation process the suspended solid was left to settle for (5 min), from the top of each beaker were collected 10 mL to read their turbidity by using a turbidity meter (Lovibond, Germany) before and after running the jar test device, turbidity removal was calculated by applying the following equation**:**

$$\text{Turbidity removal (\%)} \frac{\text{Initial Turbidity (NTU)} - \text{FINAL Turbidity (NTU)}}{\text{Initial Turbidity (NTU)}} \times 100\%$$

Were,
Initial turbidity (NTU): Before operation jar test device
Final turbidity (NTU): After operation jar test device

Results and Discussion

Identification of bacterial isolates by 16S rDNA

Four isolates were identified by PCR technique to amplify 16S rDNA gene. The Polymerase chain reaction (PCR) products were observed on agarose gel under a UV transilluminator at 1500 bp approximately compared to the DNA ladder. The sequences analysis of 16S rDNA gene of the isolates identified in a previous study (Al Khafaji *et al*., 2023) table (1). Gene of 16S rDNA is used to characterize the isolates to species level and is considered a good tool for bacterial identification due to its presence in all bacteria, the function of 16S rDNA gene has a consistent function over time and 16S rDNA gene length is suitable (Al-Dhabaan, 2019; Alyousif, 2022).

Table (1): Bacterial identification by 16S rDNA gene sequence, isolates code and the identical to the type strains of NCBI

No.	Isolates code	Closet species	accession number	identity %
1	A1	*Escherichia coli* IRQBAS57	LC428294.1	99.86
2	A3	*Arcobacter cloacae* strain F26	HE565361.1	100
3	A5	*Aeromonas simiae* strain BMK1.2	KU244021.1	100
4	D4	*Exiguobacterium profundum* strain SSPZ15	MT353657.1	100

Jar test determination of flocculating activity

Four bacterial species were selected to treat kaolin contaminated water to remove turbidity, where it was found that the bioflocculation compounds of the selected bacteria differed in their ability to remove turbidity. The results of the study showed that compounds of four bacteria species can be used as flocculants to remove turbidity from water. Different parameters such as concentration, time, and speed were evaluated for their effect on flocculation activity.

1. Effect of bioflocculants doses on the flocculating activity

Various concentrations were used such as 4 ml and 8 ml to test the effectiveness of bioflocculants in removing turbidity. Where it found the result of each of the bacteria at a dose 4 ml *Escherichia coli, Arcobacter cloacae, Aeromonas simiae, Exiguobactirum profundum* that flocculating activity to remove turbidity reach to 45% ,27.19% ,41.19% and 44.28 % while the result of each bacterium at a dose 8 ml that flocculating activity to remove turbidity reach to 49.65%, 31% ,64 47% and 63.71. The results showed that the best concentration was 8 ml for removing turbidity by *Aeromonas simiae* and *Exiguobatirum profundum* which was 64.47% and 63.71% approximately as shown in figure (1).

Concentration is an important factor in determine the effectiveness of flocculation, as it was found in this study to increase the effectiveness of flocculation at a concentration of 8 ml, which is considered an ideal dose for inducing the flocculation process, but in the event of an increase in the dose of bioflocculant , it will lead to the inhibition of the interaction between bioflocculant and kaolin clay in the solution, which leads to a decrease in flocculation, according to study (Li *et al* ., 2009).

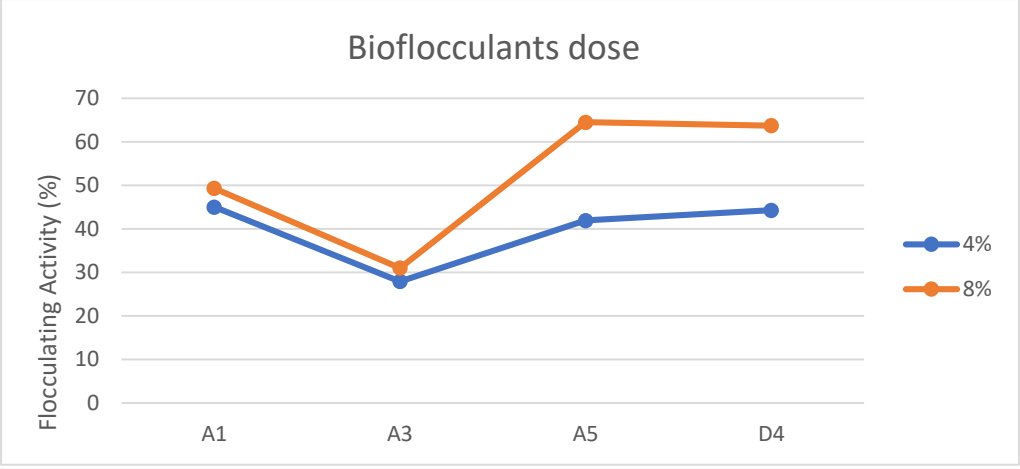

Figure1. Effect of bioflocculants concentrations on the flocculating activity of *Escherichia coli* (A1), *Arcobacter cloacae* (A3), *Aeromonas simiae* (A5) and *Exiguobactirum profundum* (D4) isolates

2. Effect of mixing speed on flocculation activity

The effect of speed was conducted by varying speed from 40 –200 rpm showing the differences in mixing speed of a solution containing kaolin clay, calcium chloride, and bioflocculants of bacterial species in a jar tester to investigate bioflocculants activity to remove turbidity. Two different time includes rabid mixing (100 rpm, 200 rpm) and one slow time (40 rpm) used. The flocculating activity result of the following bacteria includes *Escherichia coli, Arcobacter cloacae, Aeromonas simiae* and *Exiguobactirum profundum* at (100/40) speed to remove turbidity reach 47.94% ,37.1%, 66.63% and 62.89% respectively, while the result of each bacterium at (200/40) that flocculating activity to remove turbidity reach to 48%, 35.5%, 62.89% and 65.12% respectively. These results are for the best mixing speed at (100/40) which showed a flocculating activity on removal turbidity of *Aeromonas simiae* was 66.63%. The results are for best mixing speed at (200/40) which showed a flocculating activity to remove turbidity by *Exiguobatirum profundum* was 65.12% as shown in figure (2).

The effect of shaking speed on flocculation activity of bacterial bioflocculants. Speed is one of the parameters that influences flocculating activity. Mixing process during the physicochemical treatment ensures a good distribution of flocculation agents and chemical destabilization of colloids, besides it eases contact among particles and prevents damage to the formed flocks (Cros *et al.,* 2010). A study conducted by Joshi *et al*. (2019) deals with the potential of bioflocculant for the treatment of various industrial wastewaters. Bioflocculant (BFBl) produced by *Bacillus licheniformis NJ3*, showed 97% flocculating activity in primary screening (kaolin assay).

Mixing speed is an important factor that should be considered when using flocculating compounds produced by bacteria for wastewater treatment (Kurniawan *et al*., 2022). The mixing process helps distribute the coagulation agents evenly throughout the wastewater, which ensures that all of the colloids are exposed to the coagulation agents. This is important because coagulation agents work by neutralizing the charges on the colloids and bridging the gap between them, allowing them to aggregate and form flocs. The mixing process facilitates contact between the molecules of the coagulation agents and the colloids, which is necessary for effective coagulation and flocculation (Alnawajha *et al*., 2022)

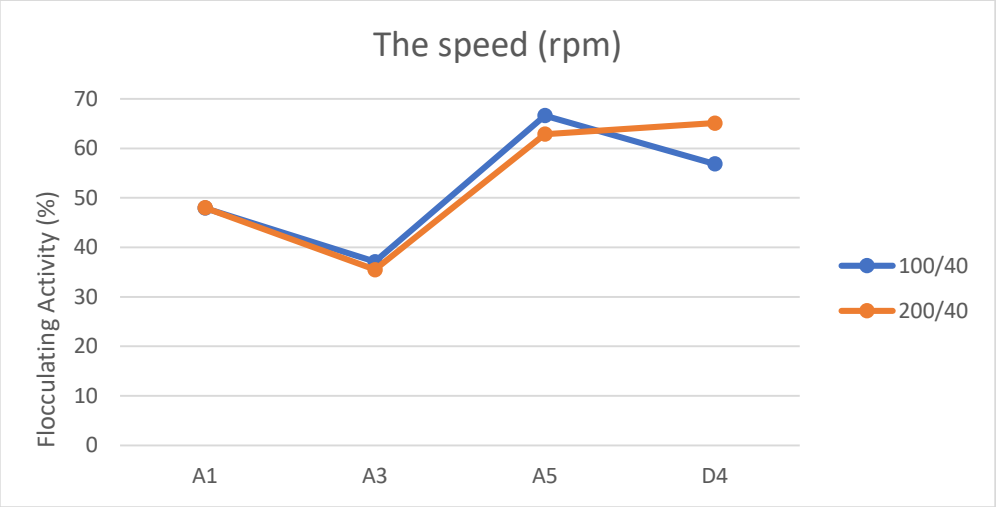

Figure 2. Effect of mixing speed on the flocculating activity of *Eschrichia coli* (A1), *Arcobacter cloacae* (A3), *Aeromonas simiae* (A5) and *Exiguobactirum profundum* (D4) isolates

3. Effect of mixing time on the flocculating activity

The effect of mixing time used such as 4 min and 6 min to test the effectiveness of bioflocculants in turbidity removal. The flocculating activity result of the following bacteria includes *Escherichia coli, Arcobacter cloacae, Aeromonas simiae* and *Exiguobactirum profundum* at a mixing time (4 min) to remove turbidity reached to 51.19%, 32.7%, 70.28% and 67.80% respectively, while the result of each bacteria at a mixing time (6 min) that flocculating activity to remove turbidity reach to 41.79%, 34.98%, 71.67% and 70.0% respectively. These results are for best mixing time at 4 min which showed a flocculating activity in turbidity removal to *Aeromonas simiae* was 70. 28% and *Exiguobatirum profundum was* 67.80% and results are for best mixing time at 6 min which showed a flocculating activity in turbidity removal to *Aeromonas simiae* was 71.67 % and *Exiguobatirum profundum* was 70.0% as shown in figure (3).

Mixing time is an important factor that affects the efficiency of floc formation. Longer mixing times generally lead to better floc formation, but it is important to avoid overmixing, as this can disrupt the flocs. The optimal mixing time will vary depending on the specific wastewater and flocculation system. It is therefore important to conduct jar tests to determine the optimal mixing time (Oruç and Sabah, 2006).

The addition of metal ions including $CaCl_2$ to kaolin suspensions during the bioflocculation process is required to induce effective flocculation by stimulate flocculation through neutralization and stabilization of the negative charges of the functional groups of the colloidal particles and the bioflocculants (He *et al.,* 2010). The bioflocculant showed an outstanding flocculating activity when Ba_2^+ was used, Ba_2^+ was able to neutralize the negative charges of the functional groups of the bioflocculant and kaolin particles, thereby shortening the distance between them, consequently resulting in a high flocculating activity (Hu *et al.,* 2022).

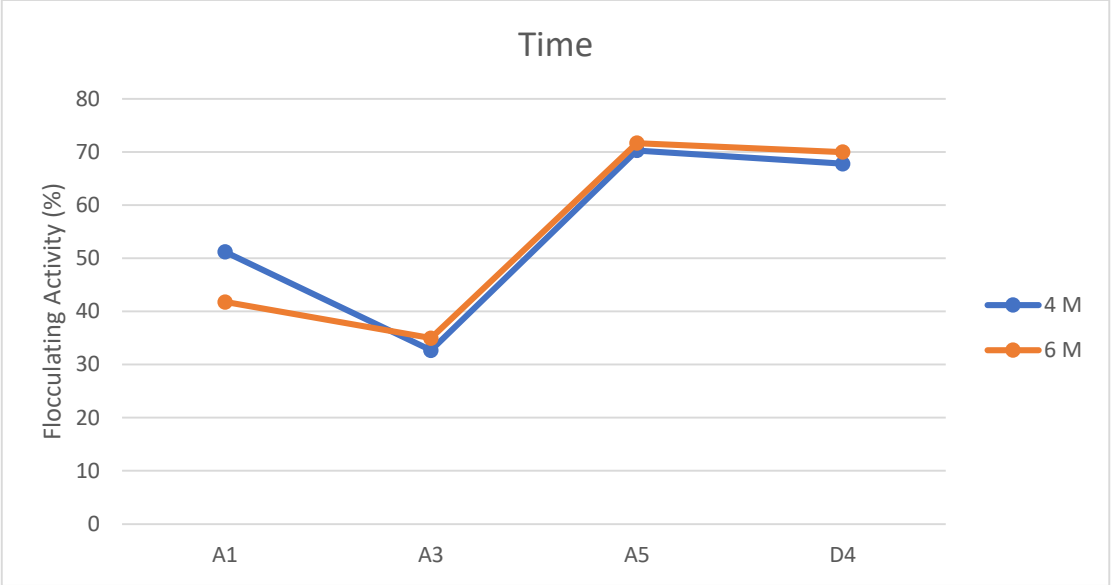

Figure 3. Effect of mixing time on the flocculating activity of *Escherichia coli* (A1), *Arcobacter cloacae* (A3), *Aeromonas simiae* (A5) and *Exiguobactirum profundum* (D4) isolates

Conclusions

Wastewater and oil-contaminated soil samples contain a variety of bacterial species that can produce bioflocculants. Four bacterial species including *Escherichia coli, Arcobacter cloacae, Aeromonas simiae and Exiguobactirum profundum* were selected that possess different flocculation activity on removal turbidity. The selected isolates were tested in different operating conditions includes bioflocculants concentration, mixing speed, and mixing time. Two bacterial species *Aeromonas simiae and Exiguobactirum profundum* were reported to have the best efficiency in removing turbidity when tested on a kaolin suspension in the laboratory.

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تقييم المواد الملبدة الحيوية المنتجة من البكتيريا في إزالة عكارة المياه الاصطناعية

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المستخلص

تقدم المواد الملبدة الحيوية عددًا من المزايا مقارنة بالملبدات الكيميائية لمعالجة مياه الصرف الصحي. فهي أكثر صديقة للبيئة وأكثر أمانًا وأقل كلفة في كثير من الأحيان. حيث بلغ نشاط التلبد الحيوي لإزالة العكارة بتركيز (0.4) 45% ,27.19% ,41.19% ,44.28% للأنواع البكتيرية الأربع *Escherichia coli*, *Arcobacter cloacae*, *Aeromonas simiae*, *Exiguobactirum profundum* على التوالي بينما عند التركيز (0.8) كانت 49.65%، 31%، 64% 47% , 63.71. وبلغ نشاط التلبد الحيوي بسرعة (40/100) لإزالة التعكر بواسطة البكتيريا التالية *Escherichia coli* , *Arcobacter cloacae* , *Aeromonas simiae* , *Exiguobactirum profundum* 47.94% ,37.1%، 66.63% و 62.89% على التوالي، بينما بلغ نشاط التلبد عند السرعة (40/200) للأنواع البكتيرية الأربع 48%، 35.5%، 62.89%، 65.12%. كان نشاط التلبد عند زمن الخلط (4 دقائق) لإزالة العكارة 51.19%، 32.7%، 70.28% و 67.80% بينما في 6 دقائق كان 41.79%، 34.98%، 71.67% و 70.0%. أظهرت النتائج أن بكتريا *Aeromonas simiae* و *Exiguobactirum profundum* لهما أفضل كفاءة في إزالة العكارة عند اختبارهما على الماء العكر الاصطناعي في المختبر. ولذلك فأن المواد الحيوية لها نشاط عالي لمعالجة مياه الصرف الصناعي.